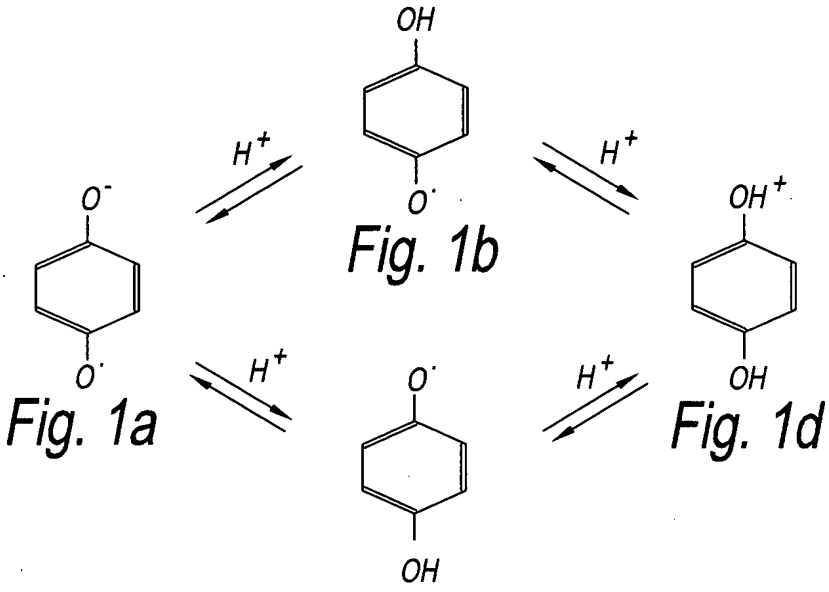


Fig. 1a

Fig. 1b

Fig. 1d

Fig. 1c

Mesomeric forms of p-benzosemiquinone. (a) anionic; (b),(c) neutral; (d) cationic.

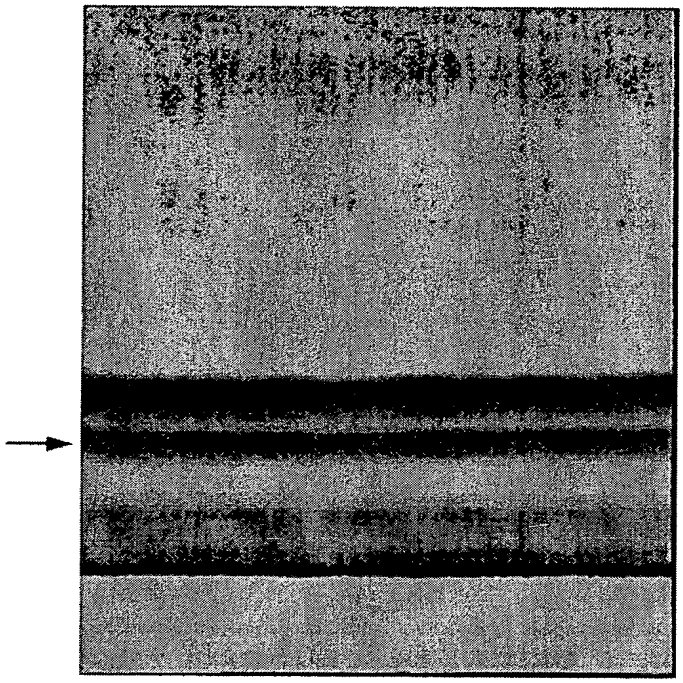


Fig. 2

Band thin layer chromatography of the methanol solution after lyophilization (step 5) ⟶ Indicates the band of the cs-oxidant.

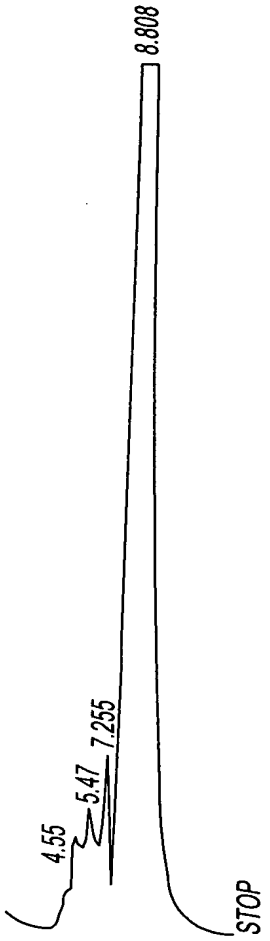
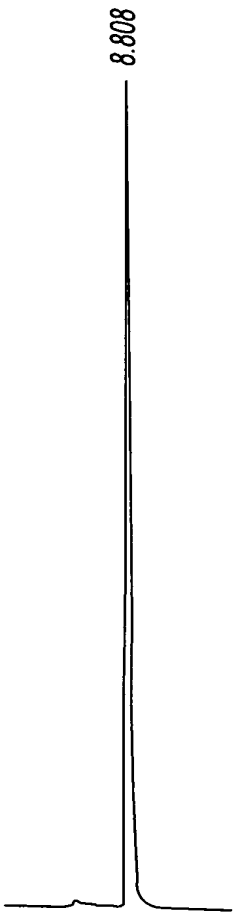


Fig. 3

HPLC profile the butanol extract after TLC. The cs-oxidant (step 6) eluted as a major peak at the retention time of 8.808 min. The amount of cs-oxidant eluted was ≈ 12 μg.



CHROMATOPAC	C-R6A		
SAMPLE NO	0	FILE	0
REPORT NO	35	METHOD	41

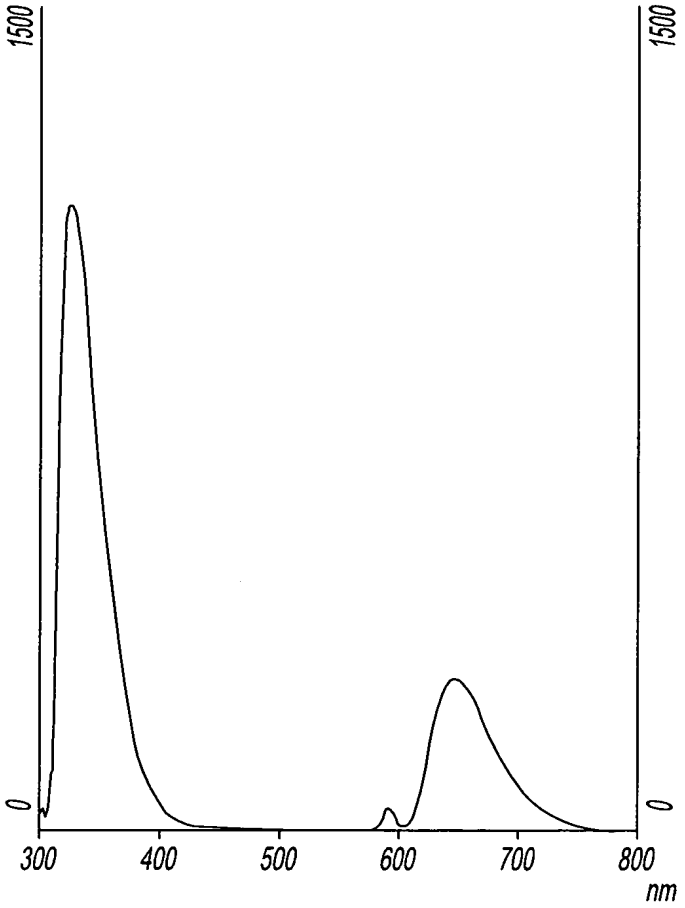
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	8.808	387815			100	
	TOTAL	387815			100	

Fig. 4

HPLC profile of the pure cs-oxidant, eluted at the retention time of 8.808 min.

Fig. 5

Thin layer chromatography of the pure cs-oxidant (R_f = 0.26)

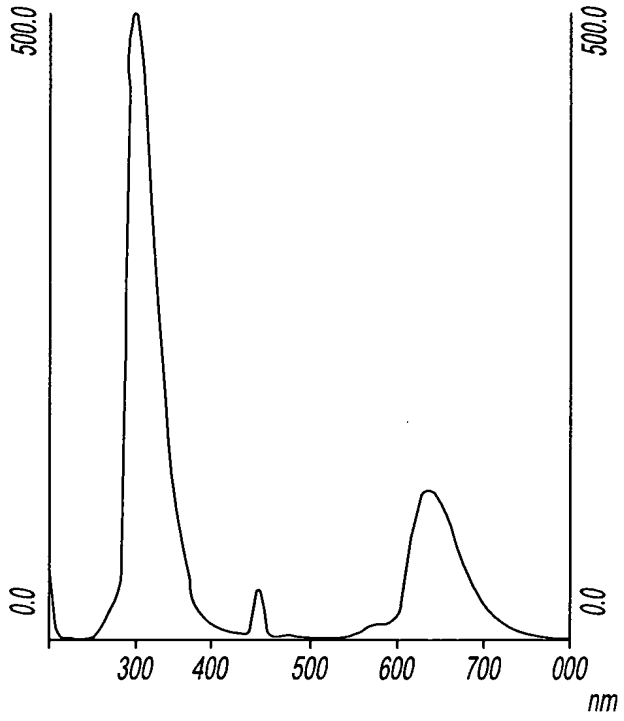


CX WAVELENGTH	293nm		
CX BANDPASS	5 nm	SCAN SPEED:	240.0 nm/min
CM BANDPASS	5 nm	RESPONSE	2 sec

NO.	PEAK		VALLEY	
1	329.6 nm	1150	299.0 nm	20.04
2	591.4 nm	44.52	560.2 nm	0.764
3	651.4 nm	201.7	603.0 nm	7.563

Fig. 6a

Fluorescence spectroscopic profile of the cs-oxidant in methanol. The excitation was at 293 nm and the emission scanning was measured from 300 nm to 800 nm. The emission maxima were at 329.6 nm and at 651.4 nm.

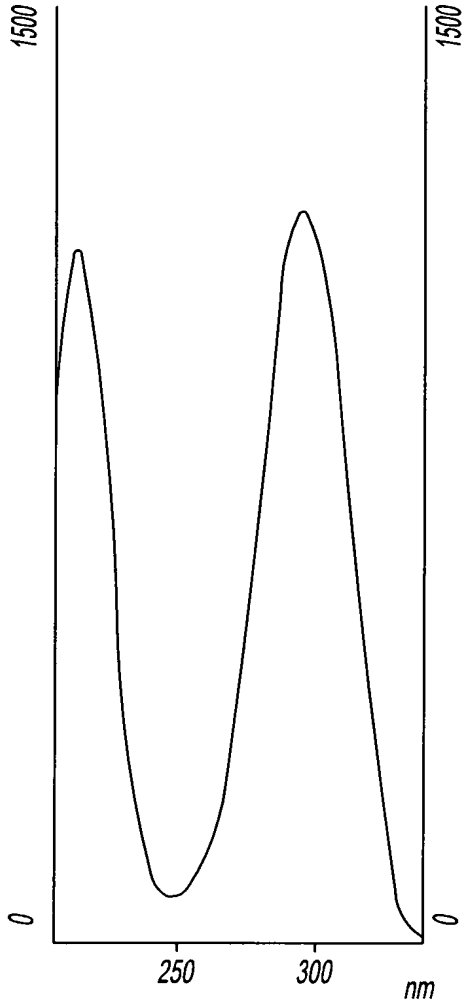


CX WAVELENGTH	224 nm		
CX BANDPASS	5 nm	SCAN SPEED:	240 nm/min
CM BANDPASS	5 nm	RESPONSE	2 sec

NO.	PEAK		VALLEY	
1	329.6 nm	502.2	261.2 nm	0.524
2	454.6 nm	41.39	228.6 nm	3.647
3	405.4 nm	3.563	476.4 nm	2.356
4	652.6 nm	121.2	527.6 nm	1.114

Fig. 6b

Fluorescence spectroscopic profile of the cs-oxidant in methanol. The excitation was at 224 nm and the emission scanning was measured from 225 nm to 800 nm. The emission maxima were at 329.6 nm and at 652.6 nm.



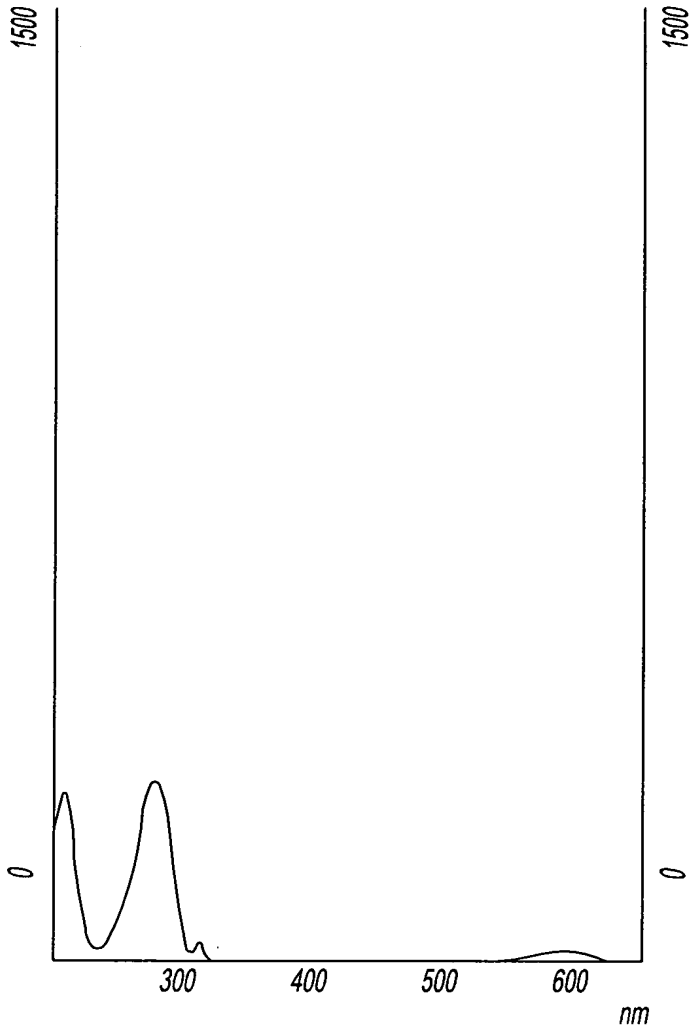
EM WAVELENGTH 330 nm
SCAN SPEED: 240 nm/min
RESPONSE 2 sec

EX BANDPASS 5 nm
EM BANDPASS 5 nm

NO.	PEAK		VALLEY	
1	228.2 nm	1115	252.4 nm	77.46
2	293.8 nm	1174		

Fig. 7a

Fluorescence spectroscopic profile of the cs-oxidant in methanol. The emission was at 330 nm and the excitation scanning was measured from 220 nm to 325 nm. The excitation maxima were at 228.2 nm and at 293.8 nm.



EM WAVELENGTH 651 nm
SCAN SPEED: 240 nm/min
RESPONSE 2 sec

CX BANDPASS 5 nm
CM BANDPASS 5 nm

NO.	PEAK		VALLEY	
1	229.2 nm	267.2	252.2 nm	19.04
2	294.8 nm	200.0	320.0 nm	7.691
3	325.0 nm	31.90	370.2 nm	0.095
4	597.0 nm	21.70	642.0 nm	0.513

Fig. 7b

Fluorescence spectroscopic profile of the cs-oxidant in methanol. The emission was at 651 nm and the excitation scanning was measured from 220 nm to 650 nm. The excitation maxima were at 229.2 nm and at 294.8 nm.

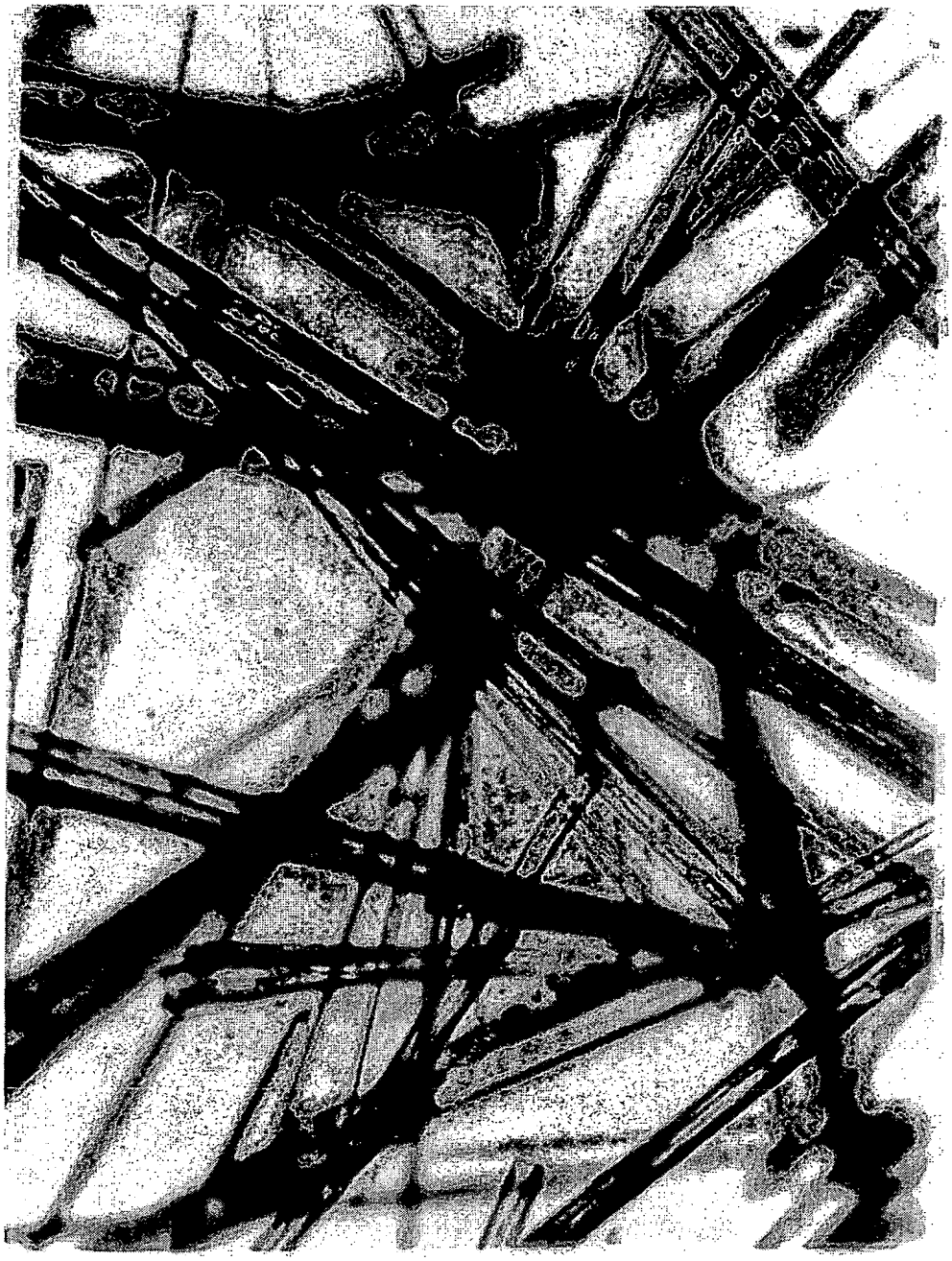
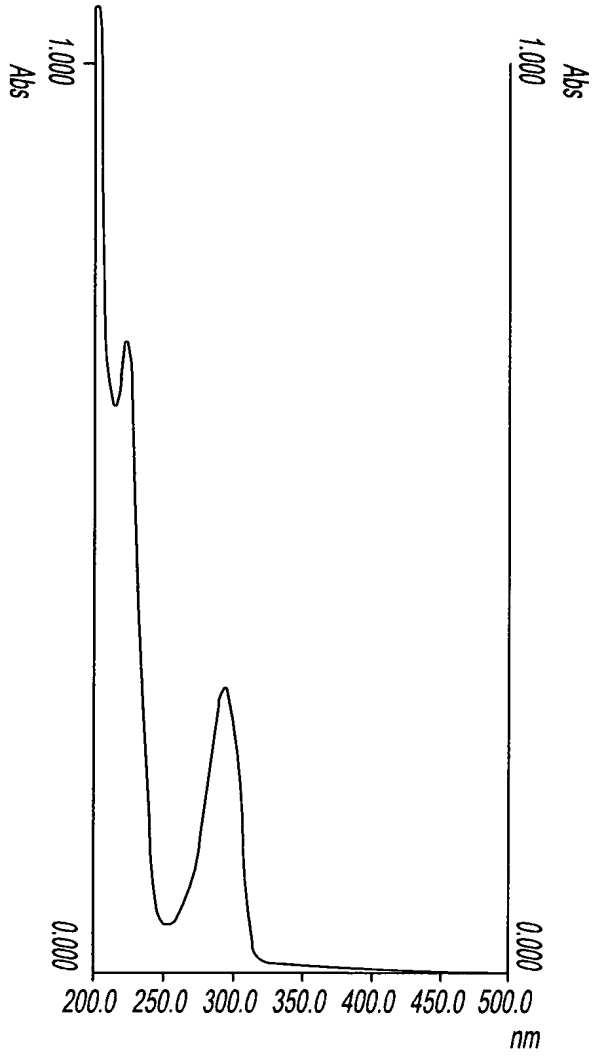


Fig. 8

Crystal structure of the pure cs-oxidant



NO.	PEAK		VALLEY	
1	293.4 nm	0.3192 Abs	250.0 nm	0.0484 Abs
2	223.0 nm	0.6994 Abs	215.4 nm	0.6261 Abs

Fig. 9

UV spectrophotometric profile of the cs-oxidant in methanol. It has two absorption maxima, one at 293.4 nm and another at 223.0 nm.

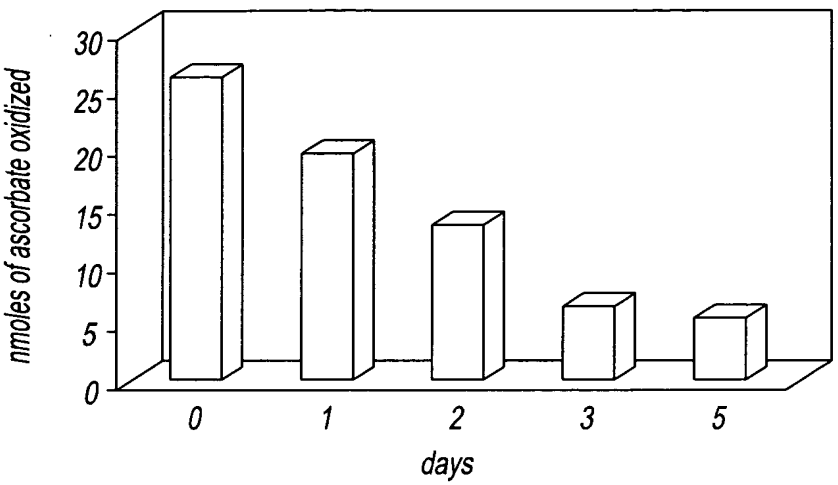


Fig. 10

Stability of the solid oxidant kept at 25°C under darkness. The stability was determined by its capacity to oxidize ascorbic acid. Ascorbic acid was mesured by HPLC analysis at 254 nm.

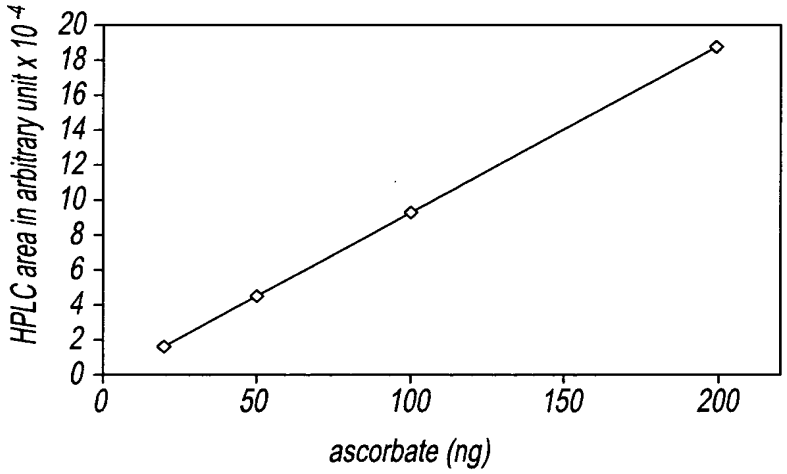


Fig. 11

Standard curve of ascorbic acid based on HPLC analysis at 254 nm.

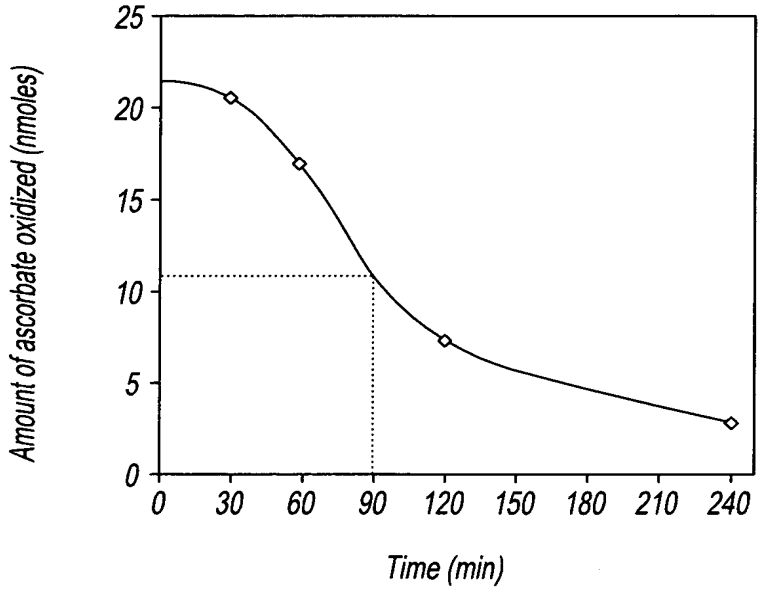


Fig. 12

Stability of the cs-oxidant in 50 mM potassium phosphate buffer at 25°C measured by its potency to oxidize ascorbate as evidenced by HPLC area.

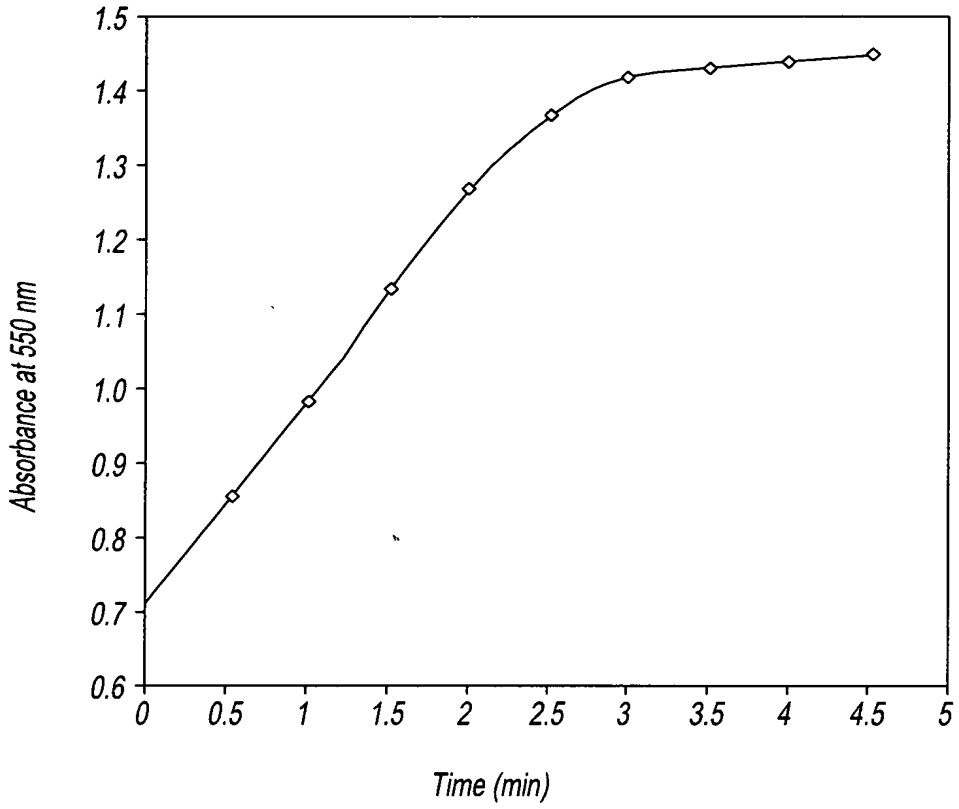


Fig. 13

Quantitative reduction of ferricytochrome c by the oxidant as measured by the formation of ferrocytochrome c with time at 550 nm. The reaction was carried out in 50 mM potassium phosphate buffer, pH 7.4, keeping the final concentration of ferricytochrome c at 100 μM. One nmole of the oxidant reduced 0.71 nmoles of ferricytochrome c.

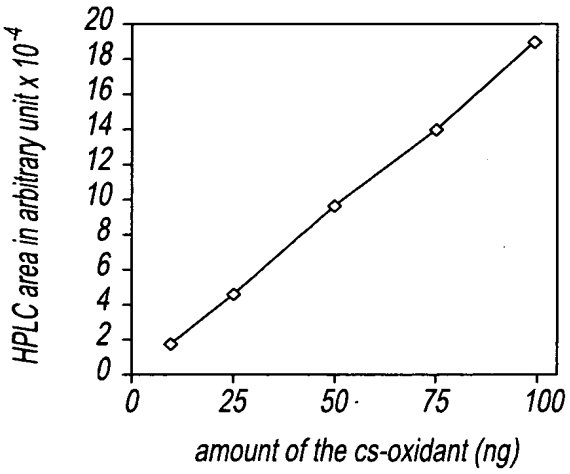


Fig. 14

Standard curve of the oxidant on the basis of HPLC area at 294 nm. Different amounts of the cs-oxidant were used ranging from 10 ng to 100 ng in 20 μl of mobile solvent.

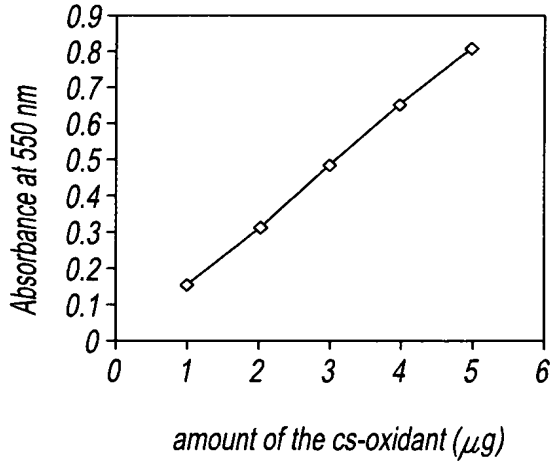


Fig. 15

Standard curve of the oxidant on the basis of reduction of cytochrome c by using different amounts of the oxidant ranging from 1 μg to 5 μg.

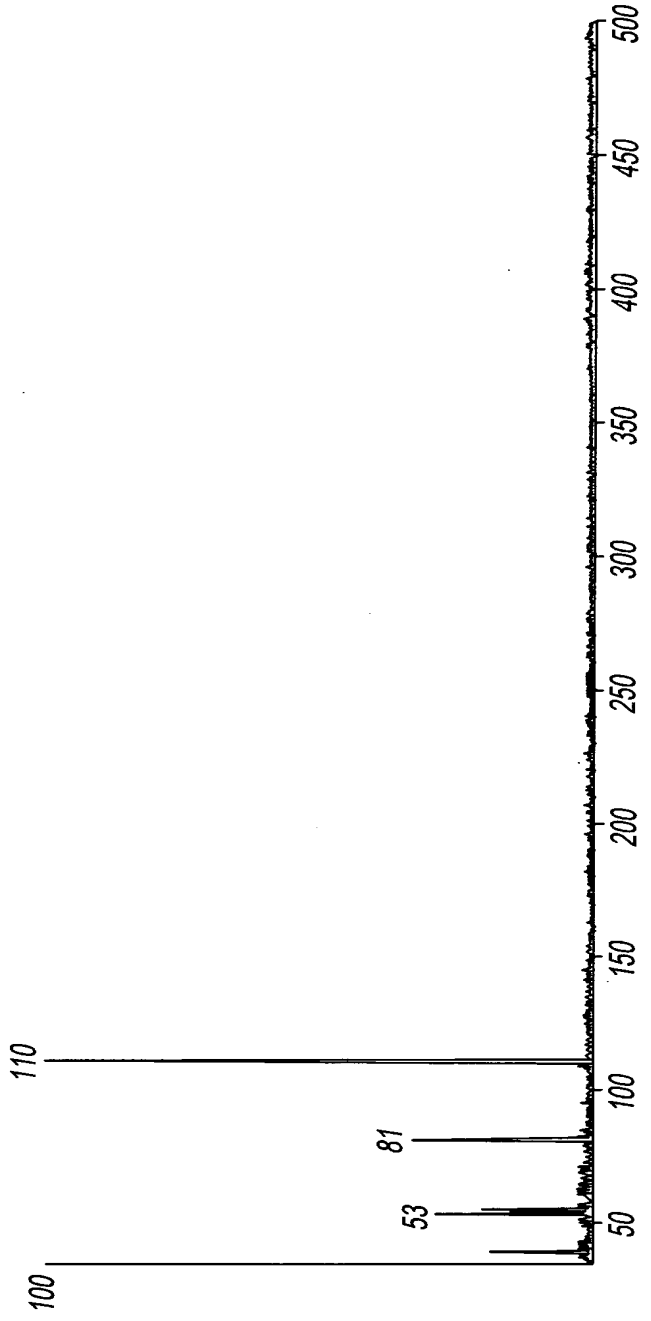
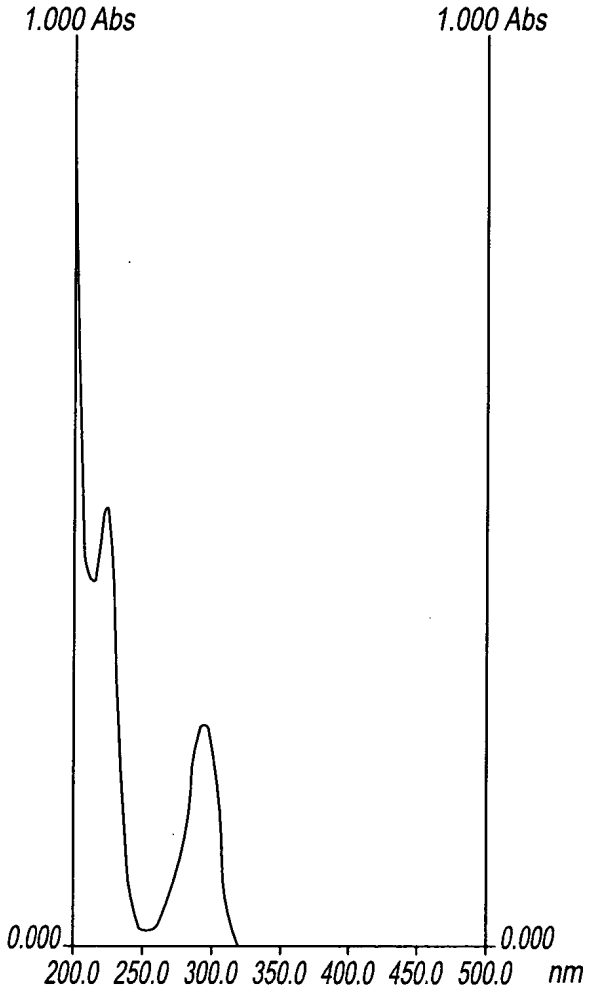


Fig. 16

Mass spectrum of the pure cs-oxidant.

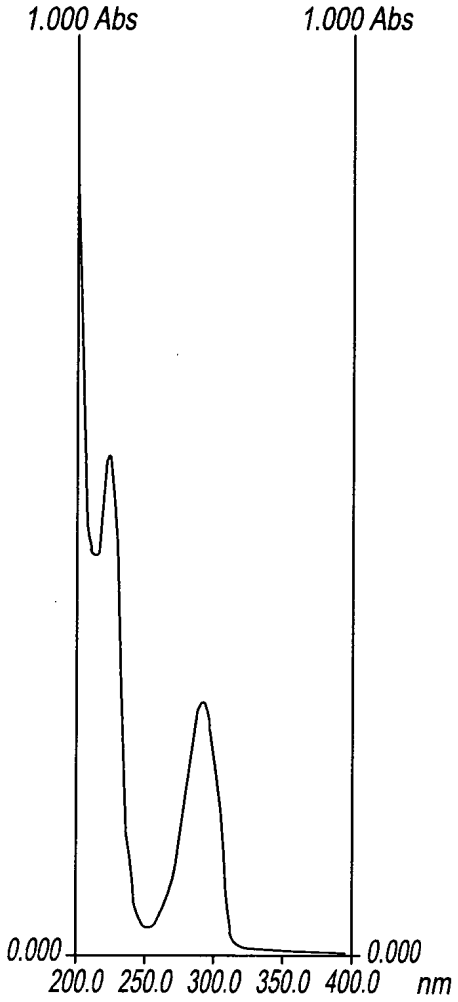


SCAN SPEED: 120.0 nm/min RESPONSE: MEDIUM
BANDPASS: 2.00nm

NO.	PEAK		VALLEY	
1	293.8 nm	0.2443 Abs	253.0 nm	0.0137 Abs
2	224.2 nm	0.4837 Abs	214.4 nm	0.3979 Abs

Fig. 17

UV spectrophotometric profile of the hydroquinone in methanol. It has two absorption maxima, one at 293.8 nm and another at 224.2 nm.

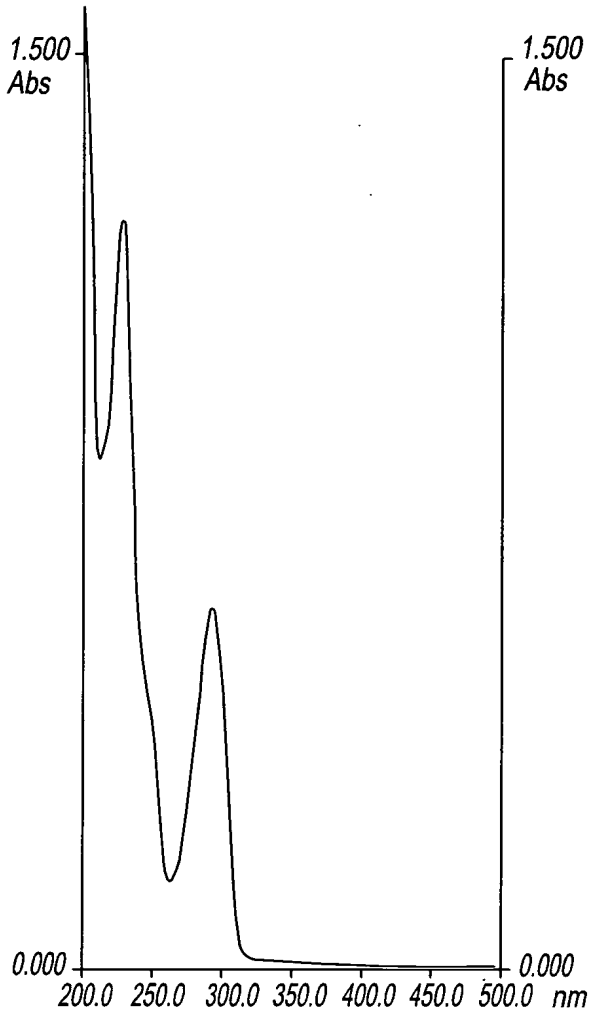


SCAN SPEED: 120.0 nm/min RESPONSE: MEDIUM
BANDPASS: 2.00nm

NO.	PEAK		VALLEY	
1	293.6 nm	0.2772 Abs	252.8 nm	0.0269 Abs
2	224.4 nm	0.5476 Abs	214.0 nm	0.4314 Abs

Fig. 18

UV spectrophotometric profile of the cs-oxidant stored at room temperature in dark for 8 days. The two absorption maxima are at 293.6 nm and at 224.4 nm.

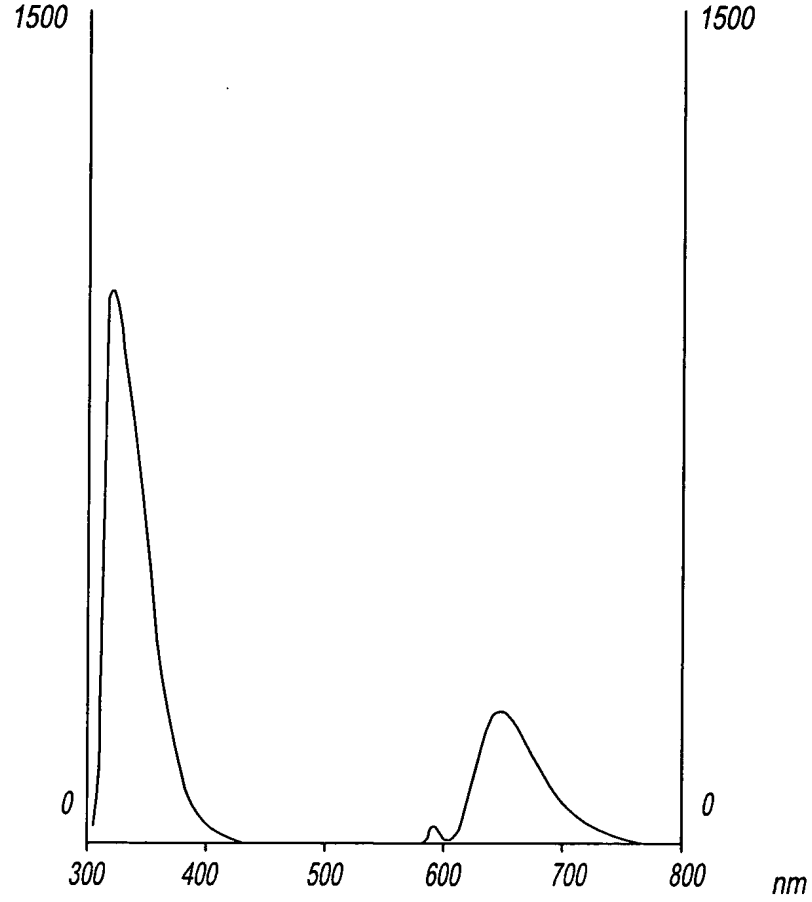


SCAN SPEED: 120.0 nm/min RESPONSE: MEDIUM
BANDPASS: 2.00nm

NO.	PEAK		VALLEY	
1	293.8 nm	0.5855 Abs	263.4 nm	0.1407 Abs
2	225.2 nm	1.2232 Abs	209.6 nm	0.8263 Abs

Fig. 19

UV spectrophotometric profile of equimolar mixture of p-benzoquinone and hydroquinone in methanol. There is a shoulder near 242 nm (the λ_{max} of p-benzoquinone).



CX WAVELENGTH	294nm			
CM BANDPASS	5 nm		SCAN SPEED:	240 nm/min
CM BANDPASS	5 nm		RESPONSE	2 sec

NO.	PEAK		VALLEY	
1	329.4 nm	1000	300.2 nm	20.76
2	593.4 nm	35.50	564.6 nm	0.477
3	651.6 nm	243.5	684.2 nm	7.546

Fig. 20

Fluorescence spectroscopic profile of the hydroquinone in methanol. The excitation was at 294 nm and the emission scanning was measured from 300 nm to 800 nm. The emission maxima were at 329.4 nm and at 651.6 nm.

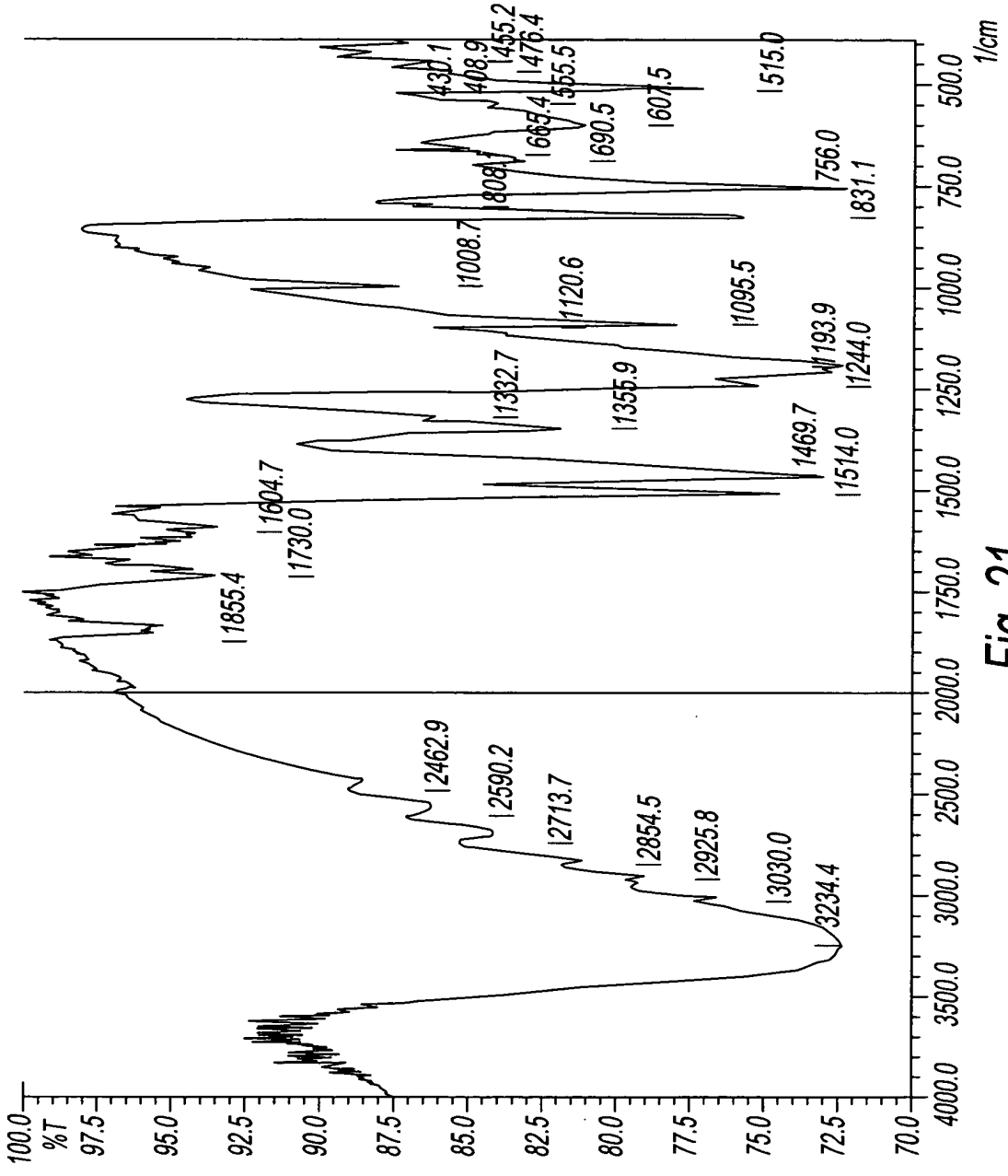


Fig. 21

FTIR spectroscopic profile of the cs-oxidant.

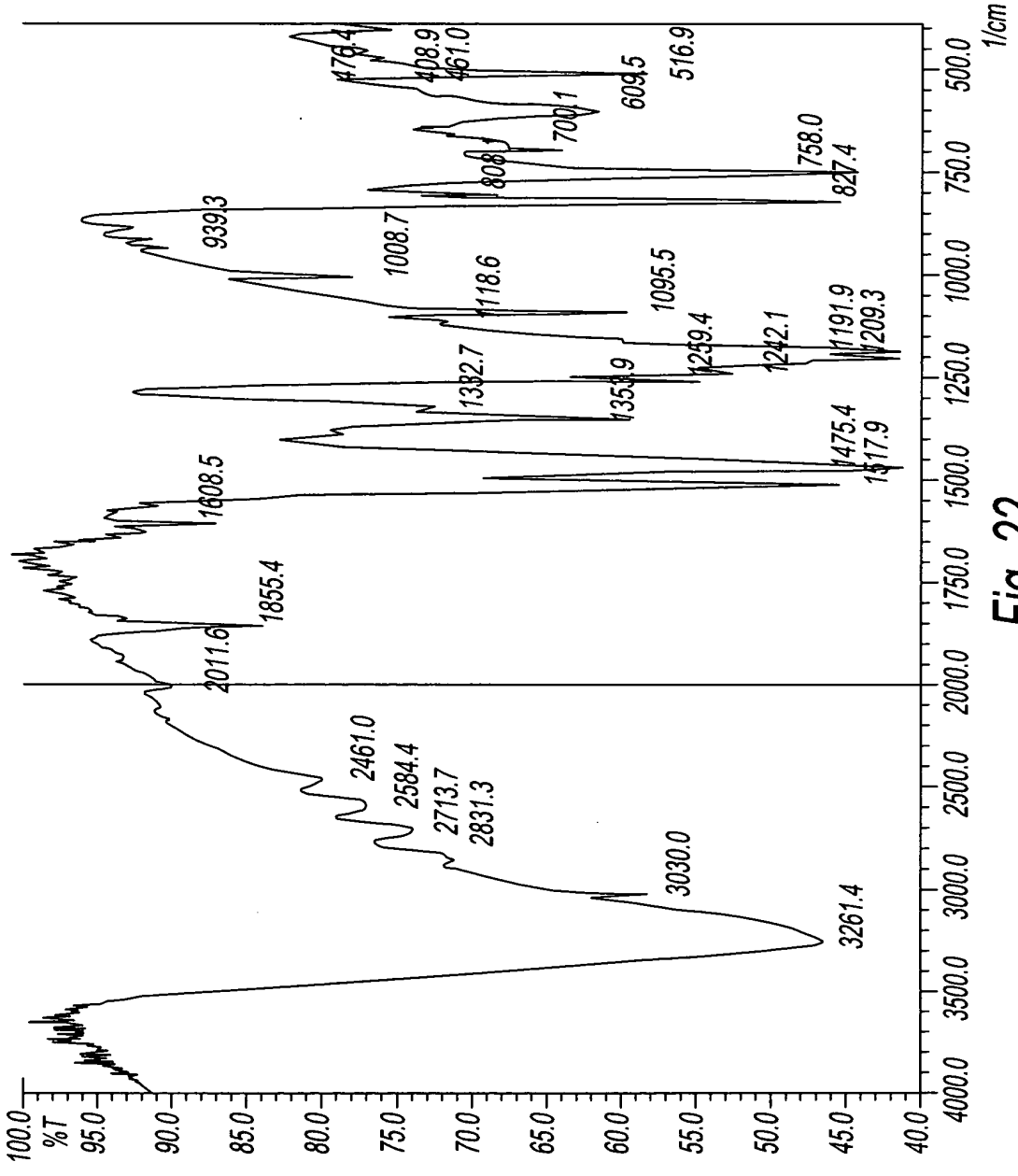


Fig. 22

FTIR spectroscopic profile of hydroquinone.

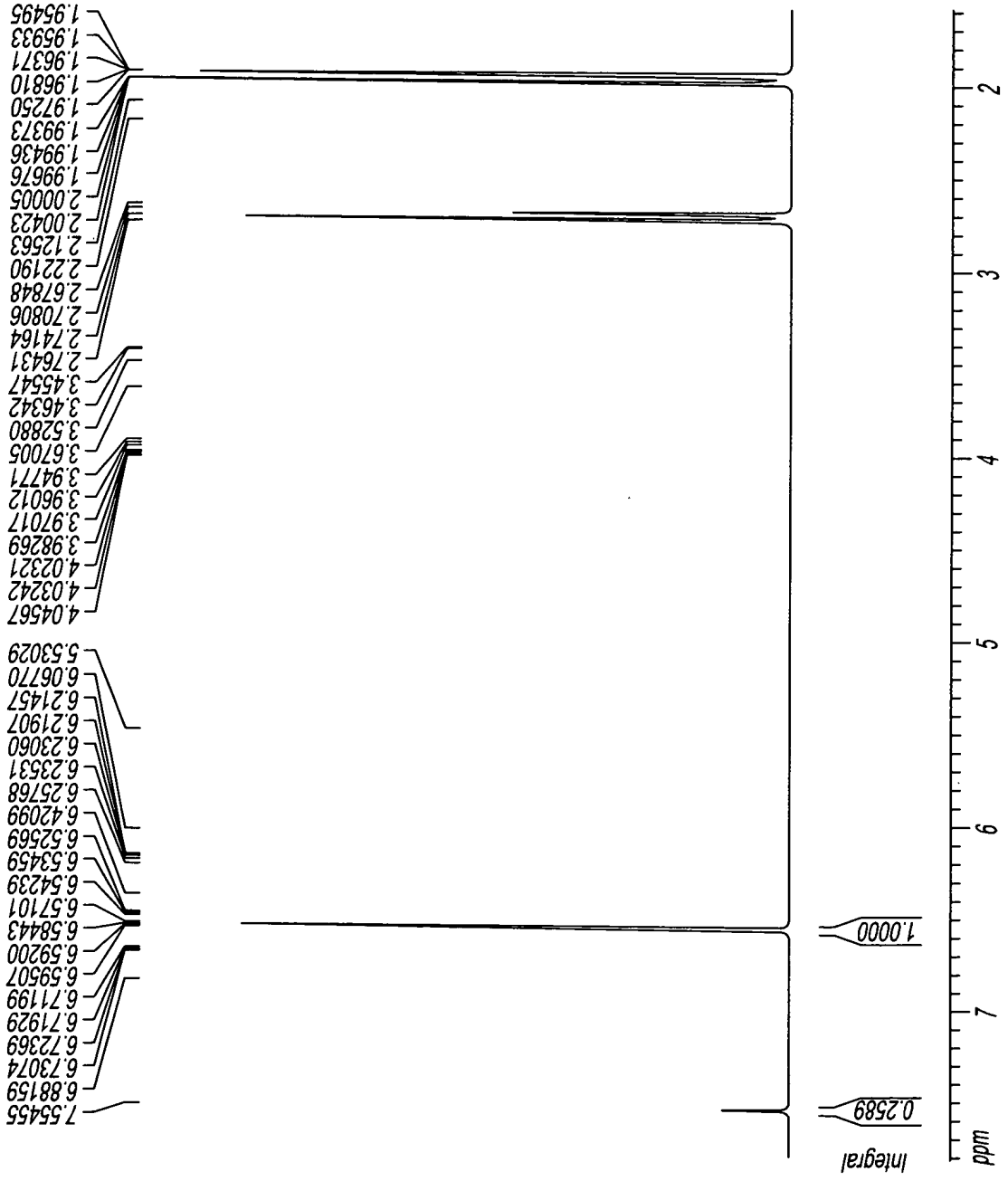


Fig. 23

H-NMR spectroscopic profile of the cs-oxidant in CD_3COCD_3.

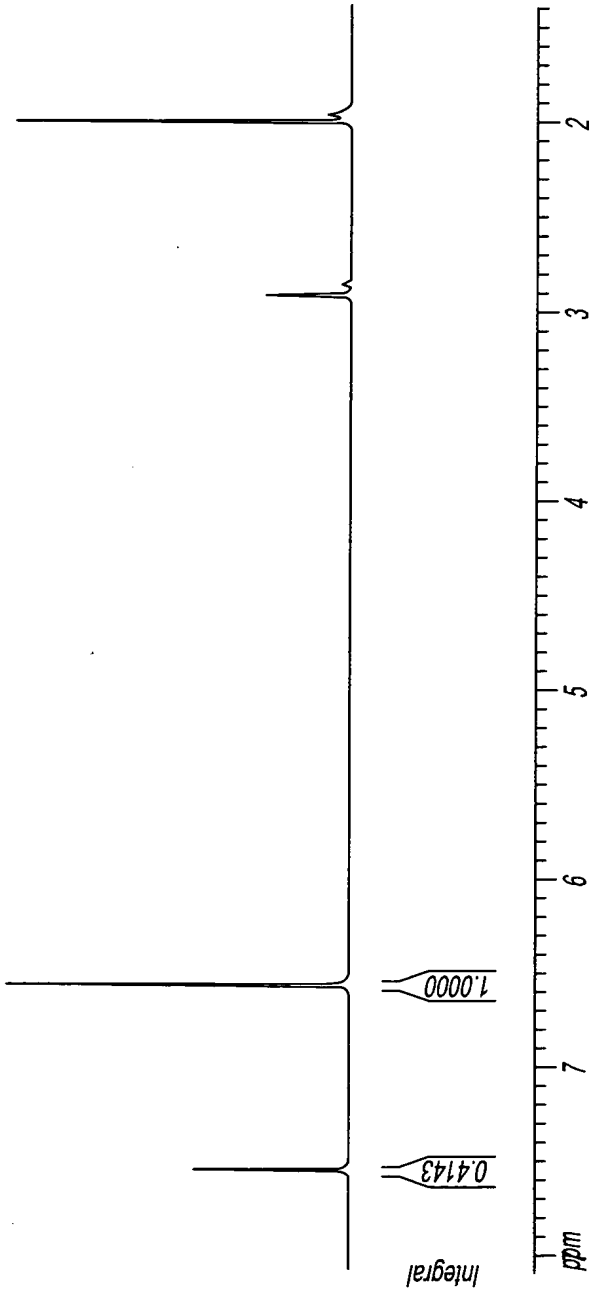


Fig. 24

H-NMR spectroscopic profile of hydroquinone in CD_3COCD_3.

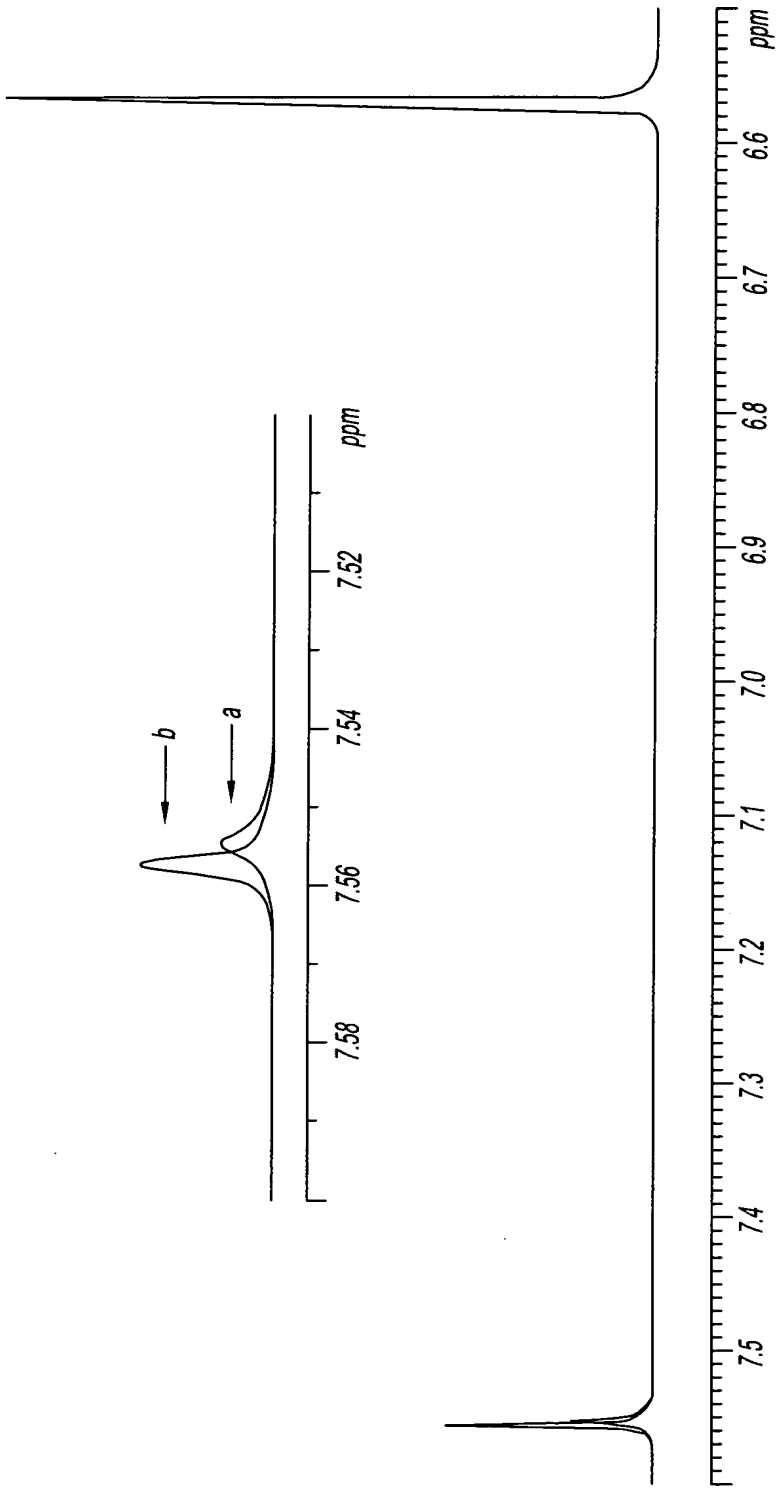


Fig. 25

Comparative H-NMR spectroscopic profiles of (a) cs-oxidant and (b) hydroquinone.

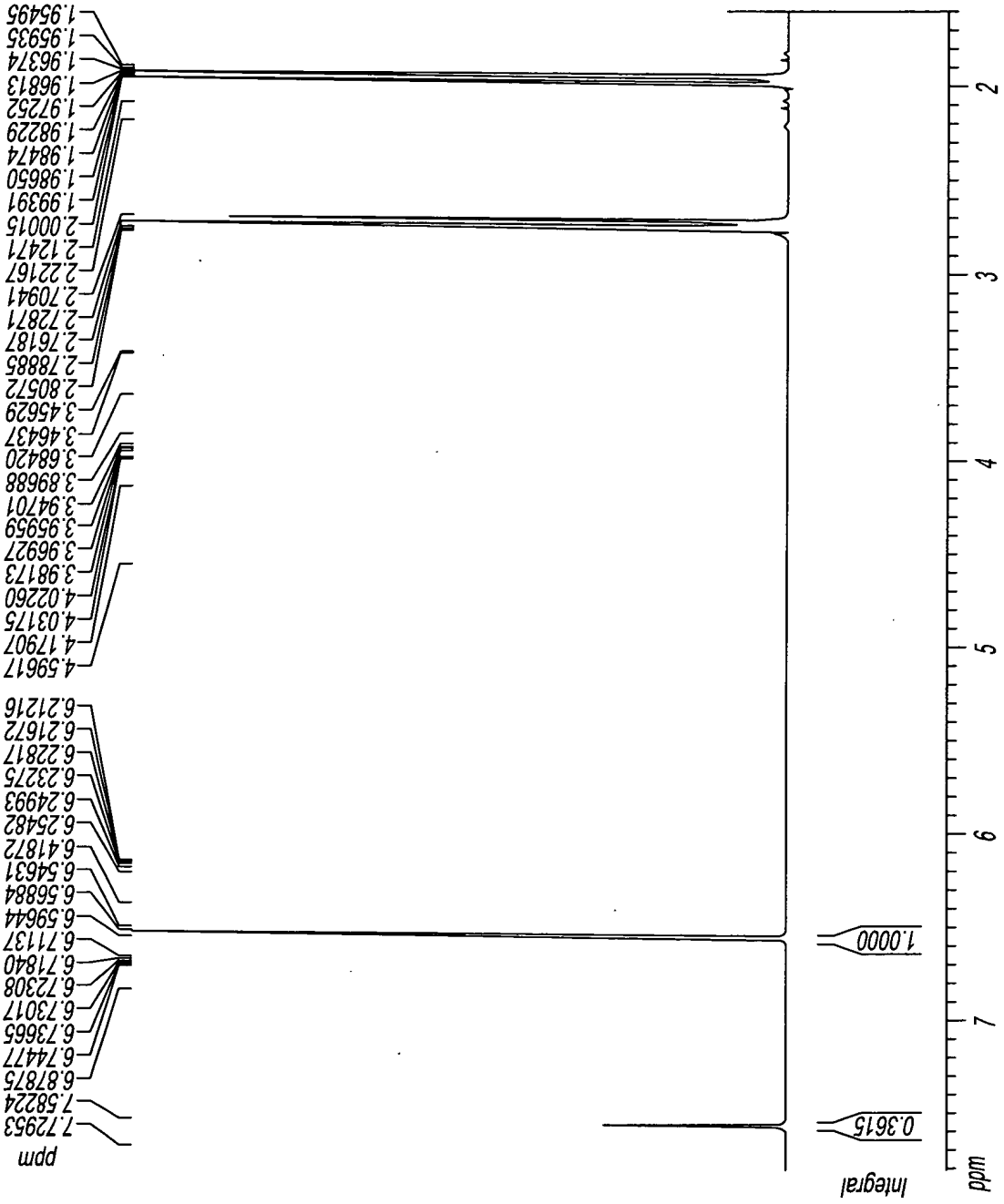


Fig. 26

H-NMR spectroscopic profile of the cs-oxidant after reduction with sodium dithionite.

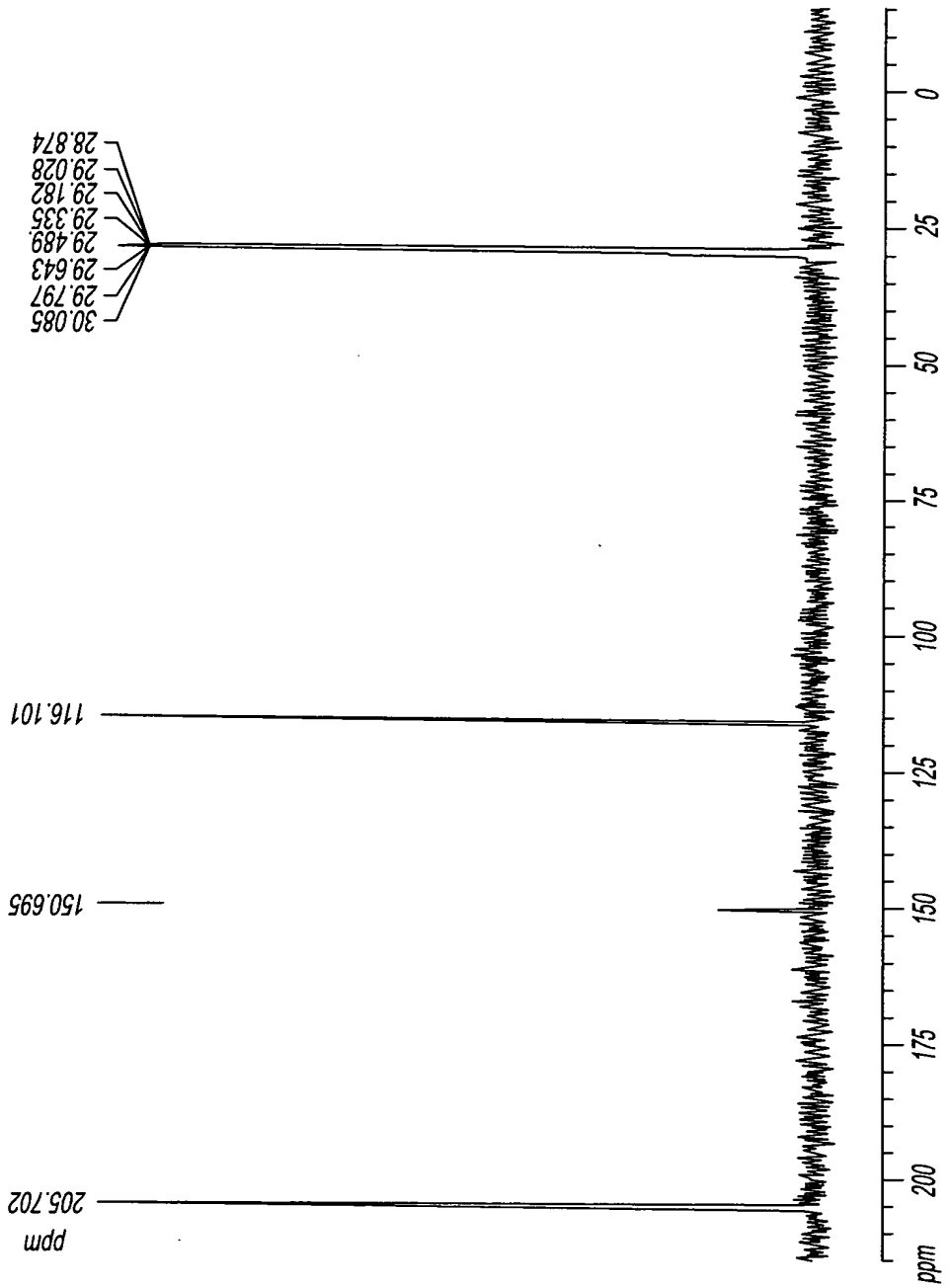


Fig. 27

C-NMR spectroscopic profile of the cs-oxidant in CD_3COCD_3.

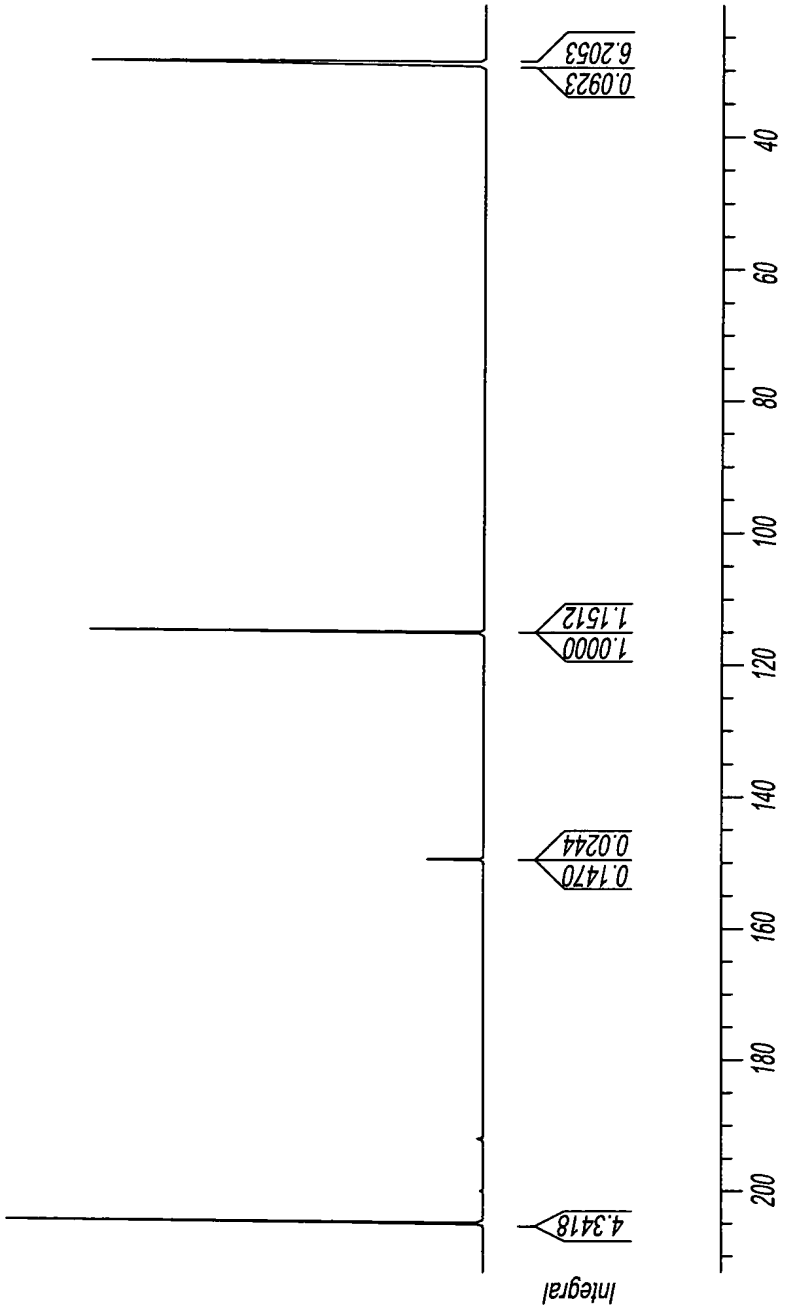


Fig. 28

C-NMR spectroscopic profile of hydroquinone in CD_3COCD_3.

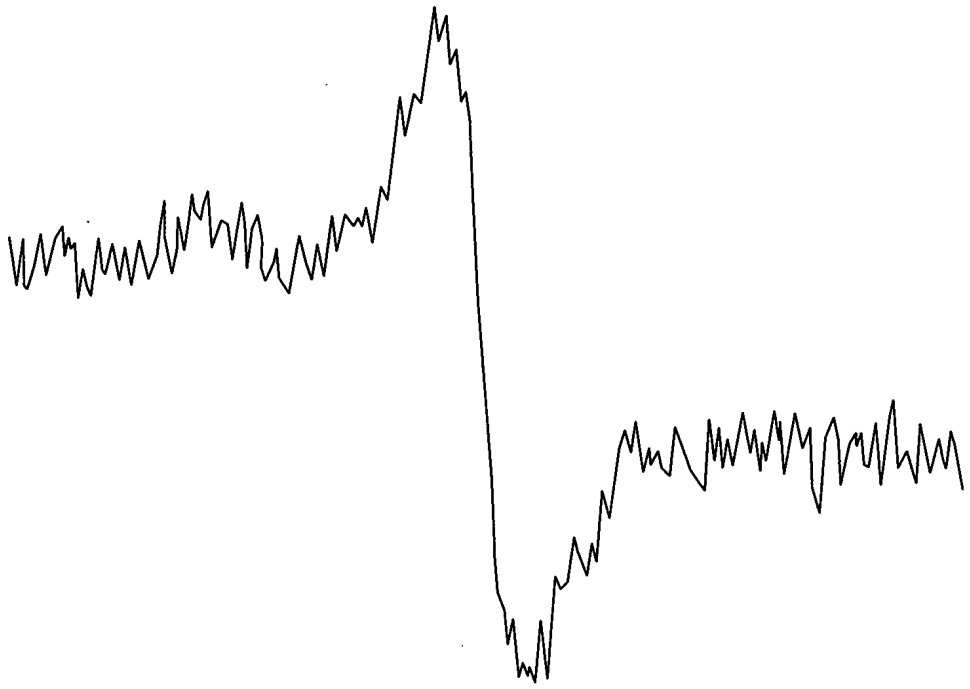


Fig. 29

Room temperature ESR spectrum of cs-oxidant, freshly prepared from 100 cigarettes. The spectrum was recorded on a JES-REIX ESR spectrometer (Tokyo, Japan). The spectral parameters were as follows: microwave frequency, 9.435 GHz; power, 2mW; field modulation width, 0.4mT; modulated frequency, 100 kHz; time constant, 0.3 sec; scan rate, 2.5 mT/sec.

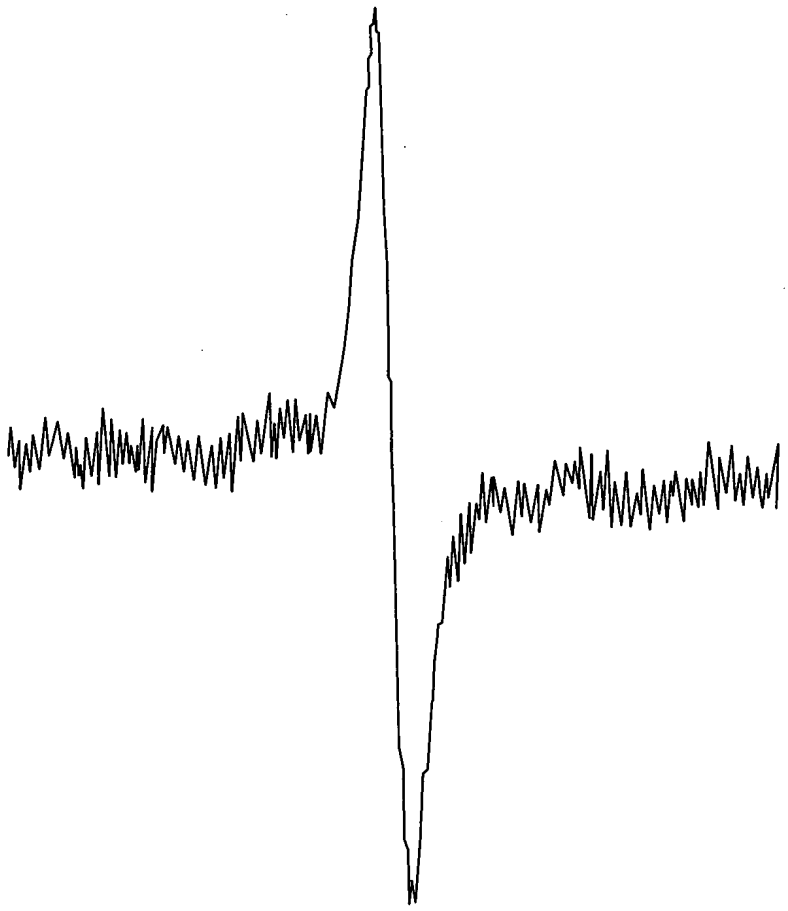
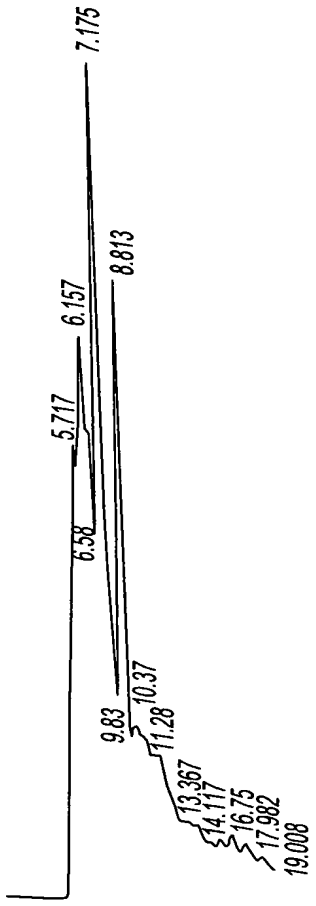


Fig. 30

Room temperature ESR spectrum of aged (10 days) cs-oxidant, prepared from 400 cigarettes.

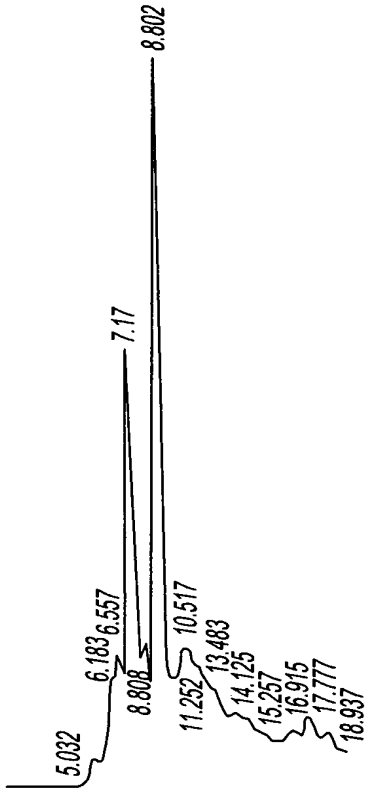


CHROMATOPAC C-R6A
SAMPLE NO 0 FILE 0
REPORT NO 48 METHOD 41

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	5.717	475376			19.0777	
2	6.157	317530	V		12.7431	
3	6.58	209664	V		8.4142	
4	7.175	708579	V		28.4366	
5	8.813	340583	V		13.6682 *	
6	9.83	99028	V		3.9742	
7	10.37	103590	V		4.1573	
8	11.28	178509	V		7.1639	
9	13.367	24236	V		0.9727	
10	14.117	15200	V		0.61	
11	16.75	9187			0.3687	
12	17.782	10303			0.4135	
	TOTAL	2491784			100	

Fig. 31

HPLC profile of the whole cs solution analyzed in the silica column (LiChrospher® Si 60, Merck).
** indicates the retention time, area and the concentration (13.6682%) of the cs-oxidant.*

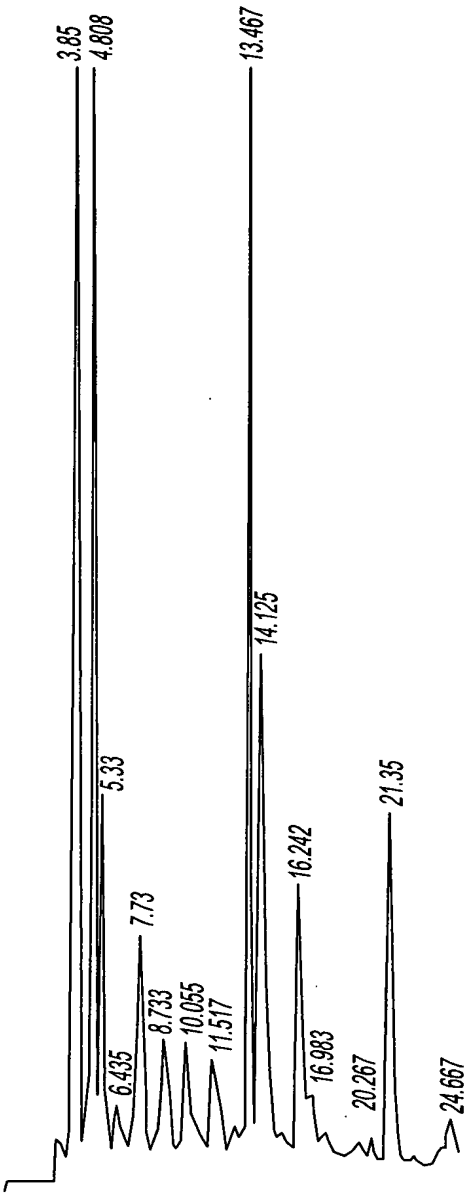


CHROMATOPAC C-R6A
SAMPLE NO 0 FILE 0
REPORT NO 47 METHOD 41

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	5.092	6469			0.5956	
2	6.183	43150	V		3.9726	
3	6.557	54830	V		5.048	
4	7.17	190600	V		17.5478	
5	8.083	59275	V		5.4572	
6	8.808	295731	V		27.2269*	
7	10.517	137178	V		12.6295	
8	11.252	129369	V		11.9105	
9	13.483	39852	V		3.669	
10	14.125	37368	V		3.4403	
11	15.257	16282	V		1.499	
12	16.915	28634	V		2.6362	
13	17.777	32483	V		2.9906	
14	18.937	14954	V		1.3768	
		---			---	
	TOTAL	1086173			100	

Fig. 32

HPLC profile of the aqueous extract of cs solution analyzed in the silica column (LiChrospher® Si 60, Merck).
** indicates the retention time, area and the concentration (27.2269%) of the cs-oxidant.*

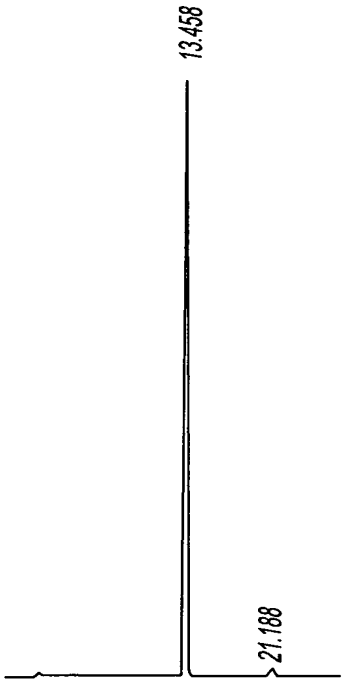


CHROMATOPAC C-R6A
SAMPLE NO 0 FILE 0
REPORT NO 8 METHOD 41

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.85	80549	V		28.8618	
2	4.808	41290	V		14.7947	
3	13.467	61513	V		28.0409	
4	14.125	45000	V		16.1239	
5	16.242	23567	V		8.4444	
6	21.35	27167	V		9.7344	
	TOTAL	279087			100	

Fig. 33

HPLC profile of the whole cs solution analyzed in the ODS column (Shim-pack CLC -ODS, Shimadzu). The cs-oxidant eluted at 13.467 min.



CHROMATOPAC	C-R6A		
SAMPLE NO	0	FILE	0
REPORT NO	9	METHOD	41

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	13.458	162863			100	
	TOTAL	162863			100	

Fig. 34

HPLC profile of the pure cs-oxidant, analyzed in the CLC-ODS column (Shim-pack CLC-ODS, Shimadzu) eluted at the retention time of 13.458 min.

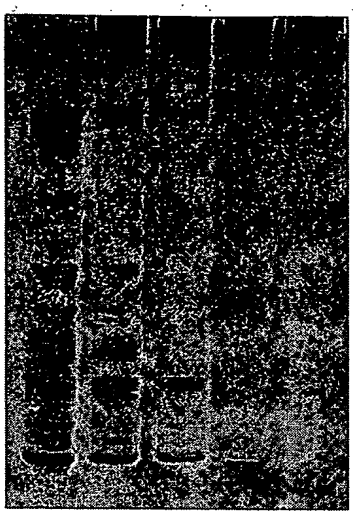


Fig. 35a

SDS-PAGE of the guinea pig lung microsomal proteins treated with whole cs solution and the cs-oxidant. Lane 1, untreated microsomes; lane 2, microsomes treated with 50μl cs solution; lane 3, microsomes treated with 100μl cs solution; lane 4, microsomes treated with 10μg cs-oxidant; lane 5, microsomes treated with 20μg cs-oxidant.

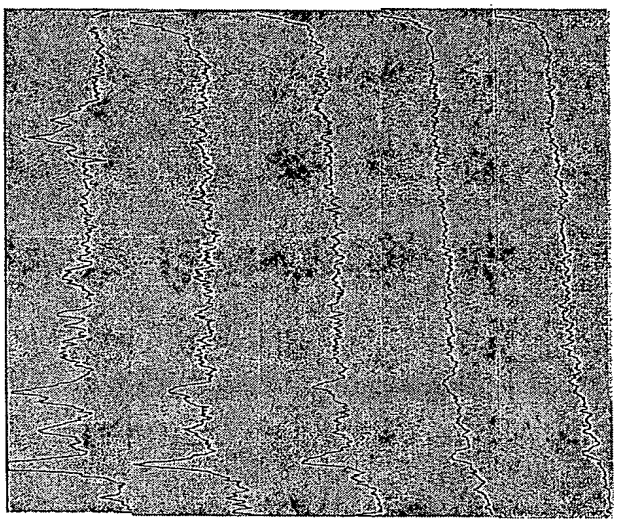


Fig. 35b

Densitometric scanning of the protein bands of different lanes as in Fig. 35a.